SECTION 5

FISH SPECIMEN PROCESSING

5.1 Introduction

5.1.1 After fish are collected, they must be either examined and identified in the field or if voucher specimens are required, they must be fixed immediately for subsequent identification in the laboratory. If the sampling crew have difficulty identifying any specimens in the field, those specimens must be fixed and later identified in the laboratory. The decision to preserve specimens should depend on study objectives. One set of specimens should be preserved during the study (especially in the early stages) so that a vouchered, archived reference collection of each species from different study areas or ecoregions will be available to investigators. The study team should be become familiar with characteristics of the specimens difficult to identify. For general purposes, formalin is usually used as a fixing agent (ASIH, 1988). This fixative solution helps retain chromatophore patterns which aid in species identification. When using formalin, care must be taken because it is highly allergenic, toxic, and dangerous to human health (carcinogenic) if used improperly.

5.1.2 If specimens are to be kept alive, they should be placed in a live well, container, or bucket and processed upon completion of sampling at each site or when the live well container or bucket are full. To minimize fish mortality in the live well or bucket, water should be changed periodically or aerated with a battery-powered pump. Fish should be handled carefully and released immediately after they are identified to species, examined for external anomalies, and weighed if necessary. Every effort should be made to minimize fish handling and holding times.

5.1.2.1 If a large number of the fish specimens are to be kept alive for later study, see Stickney (1983) for a discussion and guidelines on caring for and handling live fish.

5.2 Fixation and/or Preservation of Fish Samples

5.2.1 Fixation is the process of rapidly killing and chemically stabilizing fish tissues to maintain anatomical form and structure. Preservation is the process by which fixed tissues are maintained in that condition for an indefinite period of time.

5.2.2 Fish and ichtyoplankton should be fixed and preserved (Table 1) in the field in neutral buffered 10% formalin or borax buffered 10% formalin (a 9:1 ambient water dilution of 100% formalin) for 24 hours or longer, depending on size of fish (Haedrich, 1983, Lagler, 1956, Lagler et al., 1962, Humason, 1974, and Knudsen, 1966). The sodium phosphate monobasic and sodium phosphate dibasic, or borax, acts as a buffer which neutralizes the acidic effect of the formaldehyde. This mixture retards shrinkage in fish, prevents the hardening of soft body parts, and prevents decalcification of the tissues (Lagler et al., 1962). Fish should remain in the formalin solution for at least 1-2

weeks to fix the tissue. Fixation may take from a few days with small specimens to a week or more with large forms. Large fish or containers with closely packed fish or temperatures greater than 26.7°C (80°F) require a stronger solution of one part formalin to seven or eight parts water for fixation. Stronger solutions of formalin can cause gaping or distortion of the mouth and gills, thus care should be taken to obtain correct concentrations when making up the formalin solution (Ohio EPA, 1989).

TABLE 1. FORMULATION OF FORMALIN FIXATIVE SOLUTION

37% formaldehyde (100% formalin)	100 mL
Distilled water	900 mL
and	
Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$)	4 g
Sodium phosphate dibasic (Na_2HPO_4)	6.5 g
or	
Add one teaspoon of borax per 1/2 gallon of the formalin	

5.2.3 Since the volume of collected fishes must be taken into account upon fixation, formalin for field use should be stronger than 10%, and even 20% will not hurt. Formaldehyde gas reaches saturation in water at about 37% by weight; this saturated solution is called 100% formalin. Isopropyl alcohol and ethyl alcohol are preservatives, not fixatives. These preservatives do not fix the tissues, a necessary procedure for tissue preparation, staining, etc.

5.2.4 After fixation in the formalin, some scientists transfer the specimens to a preservative for storage. Ethyl alcohol (70-75%) or isopropanol (40-45%) preservation keeps specimens more pliable than formalin and makes working with them easier. Specimens should be rinsed in water to wash off any excess formalin, placed in a 35% alcohol wash for 2-3 weeks, switched to a 50% alcohol wash for 2-3 weeks, and placed in a 70%-75% aqueous solution of ethyl alcohol or 40-45% isopropanol alcohol for permanent preservation and storage (Haedrich, 1983; Ohio EPA, 1989). Fish should be stored in glass or plastic containers or stainless steel vats for large specimens. Metal containers should not be used. It is important that the containers be tightly sealed to prevent evaporation of the preservative.

5.2.5 Specimens are kept in tightly sealed museum jars, along with their field data. The preservatives will always modify the color, and light will further bleach the fish specimens so the various markings and colors of fish

should be documented if the specimens are to be identified later. It is advisable to store specimens in the dark at 18°C to minimize evaporation and bleaching.

5.2.6 Specimens larger than 7.5 cm should be slit on the side at least one-third of the length of the body cavity or injected with a hypodermic syringe to permit the preservative to reach the internal organs. Large and heavy fish (1-2 pounds) should also be injected in the muscles on each side of the backbone with formalin. Fish should be slit on the right side, because the left side is generally used for measurement, scale sampling and photographic records.

5.2.7 Samples for fish tissue contaminant analysis or electrophoresis must be iced, placed in dry ice, or liquid N_2 for temporary storage or shipping. Fish samples for pesticide analysis should be wrapped in aluminum foil, see Section 10, Guidelines for Fish Sampling and Tissue Preparation for Bioaccumulation Contaminants, and placed in a cooler with ice. The sample must be frozen as soon as possible after collection. Fish collected for metals analysis should be placed in plastic bags. All samples should be doubled tagged, with one tag attached outside the foil or plastic bag and one tag inside.

5.2.8 Special preservation techniques must be used for histological, histochemical, or biomarker analyses, and the investigator should be aware of such techniques before collecting tissue samples (Humason, 1974).

5.3 Labelling of Specimens in Field and Laboratory

5.3.1 Each specimen or specimens from a collecting site should be carefully labelled with at least the information asked for in the examples of labels in Figure 1.

5.3.1.1 Collection information should be both on and in the container, a tag, or a paper label. If paper labels are used, they should be made of 100% rag (waterproof) and labelled with India ink or a No. 2 soft lead pencil.

5.4 Species Identification

5.4.1 Many fish can be field identified with certainty. However, the following procedures for fish identification and verification of difficult specimens are recommended by Lowe-McConnell (1978):

1. Assemble and use the best available keys and checklists (see Section 8, Fish Bioassessment Protocols for Use in Stream and Rivers, Subsection 8.14, Selected References for Determining Fish Tolerance, Trophic, Reproductive, and Origin Classifications and Section 12, Fisheries Bibliography, Subsection, 12.5 Fish Identification).

2. Key fish to species level.

3. Maintain a voucher collection in the laboratory for comparison of specimens.

4. Verify difficult species identifications with pictures, published descriptions, known geographic range, museum and lab voucher specimens, or have the specimen identified or verified by a specialist.

FIELD SAMPLE DATA LABEL

Project _____

Date _____ Time _____ Collection No. _____

Location _____

_____

County _____ State/Country _____

Collector(s) _____

Type of sample _____ Preservative(s)_____

Method of collection _____

A. Long Form

FIELD SAMPLE DATA LABEL

Date _____ Collection No. _____

Location _____

_____

Collector(s) _____

Type of sample _____ Preservative(s)_____

B. Short Form

Figure 1. Examples of field sample data labels. A. Long form, B. Short form.

5.4.2 Scientific nomenclature of all specimens should follow the recommendations of the American Fisheries Society (Robins et al., 1990).

5.4.4 Biomonitoring laboratories should maintain a fish reference collection. Unique specimens should also added to the collection. The collection should be archived in a computer data base which cross-references field data and other pertinent information about the study.

5.5 Literature Cited

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